HISTOGENISIS OF THE HEART MUSCLE OF THE PIG IN RELATION TO THE APPEARANCE AND DEVELOPMENT OF THE INTERCALATED DISCS.

By

LUCILE WITTE

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Approved: Bennet M. Allen
Department of Zoology

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INTRODUCTION.

It is the purpose of this paper to discuss the histogenesis of the heart muscle of the pig with special emphasis upon the time of appearance, and the development of the intercolated discs. It is a much disputed question whether the intercalated discs are true cell boundaries of muscle cells or whether they are only peripheral, darker staining substances in the regular form of bands. A further aim of this paper is, if possible, to throw some light on the question stated above, by tracing carefully the formation of the discs with regard to their probable function.

METHODS.

In making this study, pig embryo and young pig hearts were used in the following series respectively, 25 mm., 38 mm., 76 mm., 89 mm., 95 mm., 102 mm., 115 mm., 120 mm., 140 mm., 165 mm., 176 mm., 182 mm., 201 mm., 238 mm., 251 mm., 277 mm., and 303 mm., and 4 months, 5 months, 8 months, and one year. Only the ventricular muscles were examined. The method of technic found most satisfactory for demonstrating the discs clearly was that employed by Zimmerman[(1)] and his students Palczewska[(2)] and Werner[(3)]. The tissues were fixed in a solution of absolute alcohol and 25% nitric acid and stained "in toto" in Grübler's Hemalum, after which they were embedded according to the usual paraffin method. Sections were cut at 5 and 8 micra.

Other methods of staining were used with no success at differentiating the discs. The iron-hematoxylin method was used on the earlier tissues with no results and the Delafield's Hematoxylin with an eosin counterstain was used on the older tissue where the discs are known to exist, with equally unsuccessful results. The striations, however, were very evident.

HISTORICAL REVIEW.

Zimmerman[(1)], Palczewska[(2)], and Werner[(3)] in 1910 worked out very complete studies of adult human and mammalian heart muscle with regard to the function of the intercalated discs and they all concluded definitely that the discs did form distinct cell boundaries. Palczewska, in her work on the human heart, evolved some diagrams (Fig. 2,9and 10) which at first glance, would seem very convincing. However, on closer study, they cannot be accepted. H. E.Jordan(4) has reviewed both Palczewska's and Werner's papers in his study of the heart muscle of the humming bird and the reader is referred to his paper for a more detailed review. Werner, in making her study of the mammals, worked out the cardiac muscle of the adult pig quite thoroughly and she noticed a striking peculiarity which was that the nuclei of the "muspelterritorien" tended to range themselves in long rows, numbering in multiples of 2 from 2 to 32, though the most usual number was 8. The intercalated discs seemed to divide these series of nuclei into distinct cells as her figures 1, 2, 3 and 4 show. She compared the ventricular muscles with those of the auricles and found that the nuclei were not nearly so numerous in the auricles ranging usually in number from 1 to 2 and 4. The discs occurred in zigzag lines in both ventricle and auricle.

Concerning the structure of heart tissue, Jordan[(4)] stated briefly that in the humming bird the muscle"is syncitial in character, the fibers anastomosing laterally and apically". A distinct membrane or sarcolemma existed and seemed to cover the entire fiber.

J. B. MacCallum[(5)] in his study of the histo-genesis of heart tissue used the pig embryo in a series of from 10 to 100 mm.. He found that there were four different types of cells in the heart of the 10 mm.. pig. One presented a network of irregular meshes in which there was a clear, unstained substance; another, a regular network; a third, a network in which some of the meshes were broken up into smaller discs by radial division; and a fourth, a network in which a single row of fibrils began to appear. The later stages of development showed an increased number of fibril bundles. The cells of 55 mm. embryo hearts were still spindle-shaped but very much lengthened and in the 72 mm.. and 100 mm.. stages the cells had lost the spindle form and had taken on almost the form of the adult muscle. The fibrils were found to be all through the cell rather than just at the periphery. MacCallum described the adult muscle of the human heart as being composed of rhomboidal cells two or three times wider than long. They sometimes broke up into branches which united with branches from other cells. The lines of union were at acute angles to the length of the fibers and each fibril of the cells sent out two or three processes which ran through these lines of union and met each other. He represented by his figure 1, a very complex structure and stated that the same structure was not to be found in other animals, but that the structures commonly known as protoplasmic bridges corresponded to his lines of union in the human heart. The cells were composed of fibrils which were dark stained and surrounded by unstained sarcoplasm. He described the fibrils with their surrounding sarcoplasm as made up of discs separated from each other by a narrow line known as Krause's membrane. He found the sarcoplasmic discs also to be divided somewhat radially and the lines of separation were continuous with Krause's membrane.

In the embryonic tissue, MacCallum noticed that the cells at the periphery of the heart were farther developed than those at the interior, indicating that the cells grow on the inside of the muscle. He considered that the development of the cells from an irregular network to cells of the adult form with numerous fibril bundles increased the heart's capacity for work and that the network of the earlier stages must be contractile.

To return to Jordan's paper on the humming bird, he gave 14 very conclusive points as to why he thought the intercalated discs could not be cell boundaries. One point which seemed very readily to contradict the cell boundary theory was that he had found discs which lay over the nucleus. They usually were peripheral in the fiber, and they also varied to a great extent in coarseness, a condition which is not common to cell walls. As has been stated, Jordan concluded from his 14 observations that intercalated discs were not cell walls or cement lines. As to the function of the discs, he only conjectured. They might have something to do with contraction, since they were found in patches and much more numerous in contracted than in relaxed fibers. His figures, however, failed to prove his statement that the discs were more numerous in contracted than in relaxed areas. Figures 2 and 4 represented discs in contracted fibers, while figures 1 and 3 represented normal areas of the fiber. According to these, there were not as many discs in contraction (Fig.4) as in relaxation (Fig 1 or 3) Figures 1 and 2 might easily be explained as discs occurring in normal relaxed fibers.

Up to the time of writing his paper, Jordan had not found discs in fetal hearts. This would seem to disprove any idea that the discs were related to the rhythmic beat of the heart. His final conclusion was that the discs "were of the same nature of the anisotropic bands, were closely related to them in position, and might represent a definite physiologic or functional state".

H. E. Jordan and K. B. Steele (6) in 1912 worked out a comparative study of vertebrate hearts in which they attempted to show that intercalated discs were to be found in animals lower than birds, and also in fetal material. In this, they succeeded, for they were able to demonstrate discs in amphibians (frogs and toads), reptiles (turtles and lizards), and fishes (trout), and in the fetal guinea pig hearts during the last week of gestation.

The technic used was Zimmermann's. The discs in the fetal hearts appeared simultaneously with the striations and increased in number with the growth of the animal after birth. The discs were also found to be present in a cat embryo of 4 days. There is some doubt here, whether this statement refers to the embryo 4 days after conception or to the animal 4 days after birth.

The lower vertebrates presented discs which were much less numerous and complex as the animals went down the scale of complexity. These discs presented no evidence in favor of the cell boundary theory, since they were superficial in position, often lay over a nucleus, were situated at random with relation to the nucleus, and did not appear earlier than the striations. They seemed to be a part of or closely related to the anisotropic lines, since they shaded into these and were parallel to them in all cases.

H. E. Jordan and J. B. Banks (7) in 1917 worked out a study of the intercalated discs in the heart of the beef. In this paper, the fetal heart was used after a study of the adult heart had been completed. The youngest fetal heart was one of approximately 2-1/2 or 3 months of age. In this, the cells were found to be fusiform, showing some signs of anastomosing laterally and terminally. Slight striations were visible and the intercalated discs appeared as large dots, shading into the telaphragma. They were situated at random in the cells but did not occur at the point of terminal fusion of two cells. None of them were more than peripheral. With the increasing age of the embryo, the discs became more distinct and more complex until in the adult heart they took the form of step-like formations very much complicated in structure.

No investigator, as far as I have been able to find, has taken up the study of fetal heart tissue in any animal with the thought of especial investigation as to the time of appearance of the discs, their development, and their function. Whatever work has been done on fetal material concerning the discs has been more or less superficial, rather than very detailed.

DESCRIPTION.

In this description, the early stages of the series of pig-embryoes will be considered briefly with regard to the histogenesis of heart muscle. The later stages will be considered more in detail since they show the origin and development of the discs.

In the 25 mm. embryo heart, the cells are spindle-shaped in longitudinal section with a large central nucleus. The cytoplasm of the cells remains clear and here and there very faint striations appear. In cross-section, the cells present the same condition that MacCallum describes. The cells seem broken up into smaller sarcoplasmic discs by walls which are continuous with the bounding membrane of the sarcoplasm. Some fibrils have appeared at the centers of these discs.

At 38 mm.. the cells have developed into a more fibrous structure. There are still some of the spindle-shaped cells to be found near the center of the ventricle, but the cells of the auricle and peripheral part of the ventricle have elongated into fibers in which the cross-striations appear more distinctly, especially at the periphery of the heart. The fibers are finer and the nuclei are still large and centrally located.

At 76 mm.. the cells seem to have lost all semblance to spindle cells, practically all through the heart. They have elongated and anastomosed until there is a complete network or syncitium of fine fibers, all of which are definitely striated. At this stage, darker portions of the striations occur at intervals, beginning at the periphery of the fiber and proceeding across at right angles to the length of the fiber. The discs, as these dark portions very clearly seem to be, do not extend across the entire width of the fiber but gradually shade into the striations or telophragma (Fig.1) In a few instances, there are faint discs extending entirely across a fiber (Fig.2).

At 89 mm. the general structure of the tissue is the same as that of the preceding stage. The discs, however, are more distinct and almost invariably extend across the fiber. They are no more numerous than in the 76 mm. stage (Fig.3) Figure 4 represents a type of disc which occurs only very rarely at this stage.

The 115 mm. embryo heart presents much more development in the discs. They are much more numerous and distinct and in every case extend entirely across the fiber. In this tissue some of the fibers are wide while others are still narrow (Fig.5). There is no change in the 120 and 140 mm. stages. The discs are numerous, straight, and occur in patches in areas devoid of nuclei. The general arrangement of the striations is different, in that some of the bands present a greater density than others. These light and dark bands occur alternately and are considered by most observers as areas of contraction. I have been unable to find any difference in the distribution of discs in this region and in other relaxed areas. There is also no marked change in the 165 mm. stage.

At 182 mm. we have the first example of a disc extending over more than two fibers. In Figure 6 the fibers anastomose terminally and the disc runs across the four fibers.

The 201 mm.. embryo heart shows the colorless cytoplasm which exists invariably as a streak through the center of the fiber in older tissue, and surrounds the nucleus. This has not been noticed in the earlier tissues, nor is it constant at this stage. The discs run to this cytoplasm but not across it. (Fig.7).

In the periphery of the heart muscle of the 238 mm.. stage, the polynuclear fibers are first noticed. The nuclei tend to arrange themselves in rows consisting of 2 or 4, rarely 3. The mononuclear state exists farther toward the center of the ventricle. The fibers are very compact and give the appearance of broad fibers. The discs take the step formation more regularly than heretofore (fig. 8 and 9).

Fig. 10 represents the discs at the 251 mm.. stage. They are quite frequent in occurrence and many of them, as here represented, are thick.

Fig. 11 and 12 represent the condition of the discs in the 277 mm.. and 303 mm.. stages respectively. There is no marked change. The risers occur more frequently and the discs often extend over two or more fibers.

In all of the tissue studied so far, the discs have increased in number by means of new and incomplete discs developing and gradually growing across the fiber. Figure 7 is an example of such discs.

There is a gap between the embryonic tissue and the post-natal, since no stages were studied between the 303 mm. pig which is very close to the age for birth and the 4 month old tissue. There are, however, no very marked changes between the two.

At five months a new feature in the discs appears. Instead of the discs extending across the fiber in a compact formation, they seem to be made up of coarser granules on each separate fibril as is shown in figure 13. Figure 14 shows three fibers which are parallel and are separated by wide clear spaces. The discs are at the same level in the three fibers, but instead of their being straight bands, they are zigzag as though the separate fibrils had been pulled back and forth till the regions of coarse granules were out of line with each other. This type of disc occurs quite frequently throughout this stage.

The year old tissue shows almost entirely the zigzag bands running across several fibers. They cannot be described as exact step-formations for they do not lie parallel to the telophragma (Fig 15 and 16). Those discs which are straight are short. The type of discs and the arrangement of the nuclei in the tissue is just as described by Miss Werner. However, I cannot verify her statement or her drawing showing that the nuclei are bounded at either end of the series by discs. If the discs were visible at one end of the series of nuclei, they were not at the other end.

The year old tissue, then, represents the limit reached in this study, where the discs extend over several fibers at nearly the same level, or go up and down in a series of "steps" and "risers". These last represent the most complex of the discs found.

DISCUSSION.

As regards the early embryonic structure of the heart muscle, I have been able to verify J.B.MacCallum's statements. The early tissue exists as spindle-shaped cells and it is very evident how the individual cells gradually anastomose and form the fibers of the adult heart tissue. MacCallum contends that these spindle-shaped cells gradually lengthen and branch and unite with each other at the ends of the branches by definite lines of demarcation. He adds that these lines of demarcation correspond to the protoplasmic bridges found in tissues of other animals described by some investigators.

Thus MacCallum places himself on the side with Zimmermann and his students and contends that adult heart muscle does not become truly syncitial but remains cellular with the lines of demarcation, or discs, as I shall call them, representing the walls between the cells.

In my study of this change from cellular to fibrous tissue, I cannot show that the discs form cell walls. They are too irregular in position and too infrequent in occurrence at such an early stage.

Observations show that striations and discs do not appear simultaneously in the heart tissue of the embryonic pig. The striations appear first. These facts need not contradict what Jordan and Steele said for they studied the embryonic heart of the guinea pig and cat. Nor should they contradict Jordan and Banks' statement that the discs appeared in the heart of beef simultaneously with the striations. The other work done on fetal material with regard to the discs has been more or less superficial. However, it may be true that the time of appearance of the discs varies widely among the different animals.

It cannot be said that the discs in the embryonic pig's heart are cell walls or cement lines. In the first place, they are only peripheral for they pass out of focus very readily. Nowhere in the tissue is it possible to keep the discs in focus through the entire thickness of the fiber. Also, they occur so many times in rather close proximity with no nucleus between them, as is shown in Figures 5, 7, 9, 10 and 11. The discs do not present a different structure in the older material except that they are more complex and occur more frequently in step-like forms. They are still peripheral in the 5 month tissue and they are very distinctly darkened, portions of each fibril arranged in the form of a band, rather than extending across the fiber as a connected disc. (Fig.13 and14). These facts would disprove what Zimmermann, Palczewska, and Werner concluded concerning the inter-cellular nature of the discs.

Jordan presents the theory that the discs may be functional in connection with the contraction of the heart. From the facts observed in this study, it seems improbable that the discs would be caused by the contractions of the heart muscle, or would result from the rhythmic beat of the heart. The striations do not appear till the heart has attained definite form, sometimes after the beating of the heart begins. The discs do not appear till sometime after the striations, so that any theory that discs were formed at contraction or with the beginning of contraction of the heart would be disproved.

It may be that the discs develop physiologically for the purpose of strengthening the muscle fibers. They appear shortly after the striations and at about the time the muscle cells begin to anastomose and form fibers. The fact that the discs become more numerous and complex with the increasing growth and activity of the heart seems to strengthen the theory mentioned above.

S U M M A R Y.

1. The early heart tissue of the pig is cellular in structure, composed of spindle-shaped cells which later anastomose terminally and laterally to form a network of fibers.

2. The striations appear earlier than the discs but only here and there throughout the tissue.

3. The discs appear at the 76 mm. stage, much earlier than in any other animal studied, with the exception of the cat embryo of 4 days.

4. The discs are at first dots or incomplete bands beginning at the periphery of the fiber and growing across. With the advance in development, they become straight discs across the entire fiber, then across two or more, and finally assume the more complex type of discs and "risers".

5. The discs do not appear more numerous in contracted areas, than in relaxed areas.

6. The discs are not to be found at either end of a sèries of nuclei, thus forming a cell, for they are almost invariably in close proximity to each other, occurring in patches, and seldom is a nucleus to be found between them.

7. The theory is put forth that the discs serve as strengthening bands in the muscle fibers, since they appear at about the time of the change from cells to fibers, and increase in number and

complexity with the growth and activity of the heart.

In conclusion, I wish to acknowledge my indebtedness to Professor W. J. Baumgartner for his aid in obtaining material and for his very helpful suggestions and criticisms.

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DESCRIPTION OF PLATES

The illustrations were made with the aid of the Bausch and Lomb camera lucida.

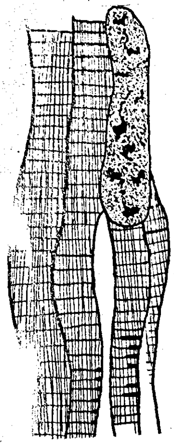
All figures are of tissue fixed in the alcohol nitric-acid mixture and stained in hemalum according to Zimmermann's technic, and are magnified 3500 diameters. Except 17 & 18 which are The original magnification is reduced one-fifth in reproduction.

~~The drawings were made with water-proof India ink.~~

PLATE I.

EXPLANATION OF FIGURES.

1. Fibers from ventricular tissue of 76 mm. pig embryo, in which the discs first make their appearance. The bands here are short or merely granular dots in line with the anisotropic bands.
2. Portion of fiber from 76 mm. pig embryo showing a completely developed disc.
3. Type of disc commonly found in an embryo of 89 mm.
4. A disc taking the step formation, only rarely found in the 89 mm. stage.
5. Fibers from heart muscle of the 115 mm. pig showing the variable width and the larger number of complete discs.
6. Fibers showing terminal anastomosing and a disc running across all four fibers. (182 mm. embryo).
7. Fibers showing the definite unstained cytoplasm which surrounds the nuclei in older heart tissue almost invariably. Also developing and completed discs are shown which explains how discs become more numerous (201 mm).
8. A definite example of the step and riser found in 238 mm. heart muscle.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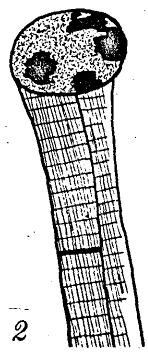
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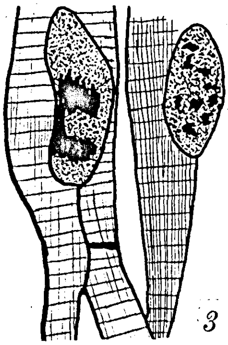
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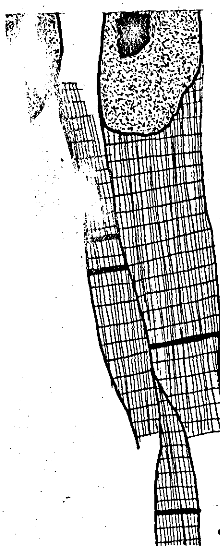
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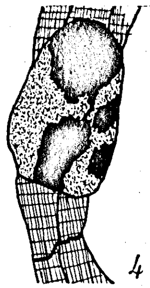
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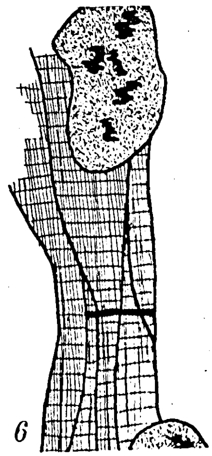
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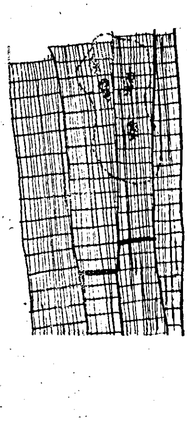
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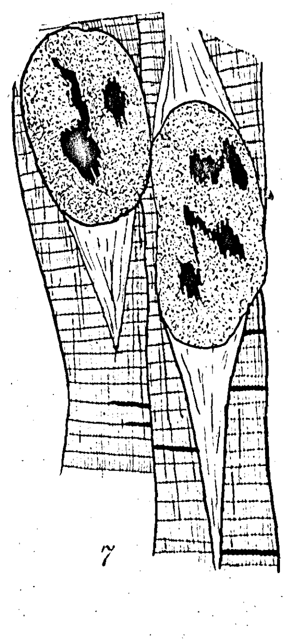
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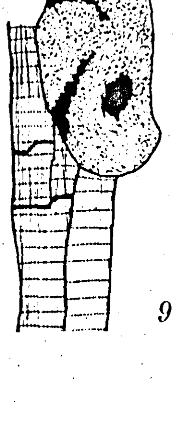
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PLATE II.

9. Another example of the "step" and "riser" forms of discs found in 238 mm. heart muscle.

10. Discs from the 251 mm. stage. They are thicker at this stage than in the earlier stages.

11. Fibers from the 277 mm. stage, showing a typical grouping of discs.

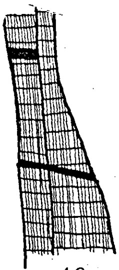
12. Fibers from the 303 mm. stage showing a more developed "step" and "riser".

13. Fibers from 5 month tissue in which the discs appear as granules laid down on the separate fibrils.

14. Three parallel fibers from 5 month tissue in which a peculiar type of zigzag disc occurs.

15 and 16. Zigzag discs as found in year old tissue. These are very characteristic of this tissue.

17 and 18. These figures were drawn from 277 mm. tissue at a magnification of 2000 diameters, to show a larger area and thus show the comparison between the number of discs in a contracted area (17) and a relaxed area (18).



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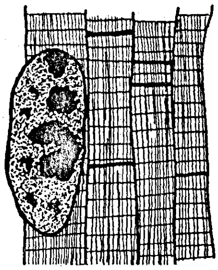

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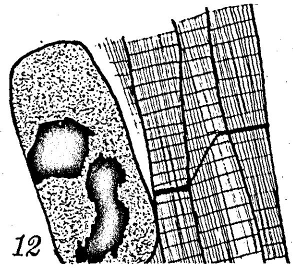

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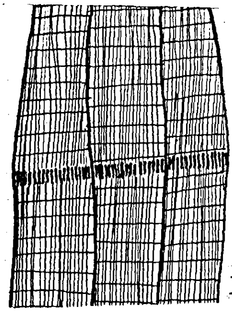

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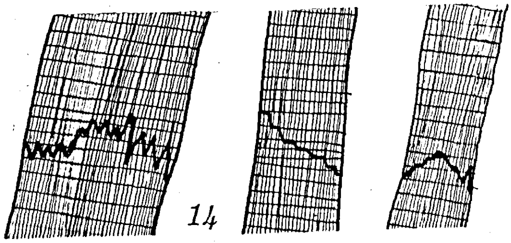

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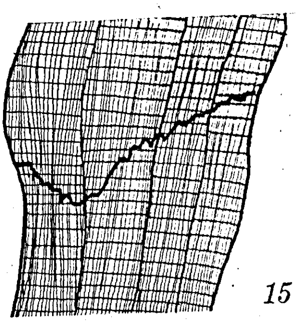

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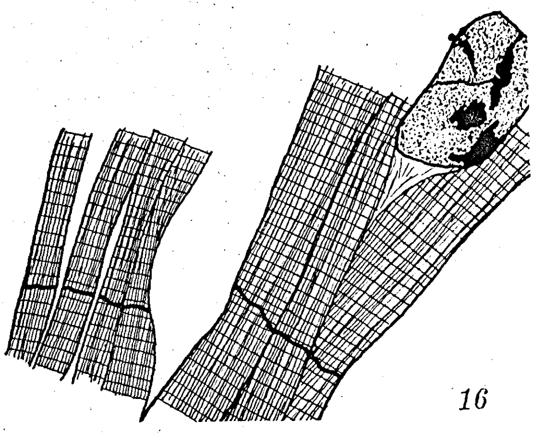

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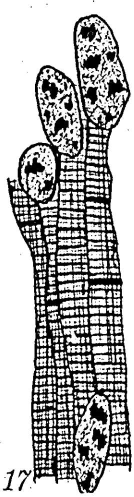

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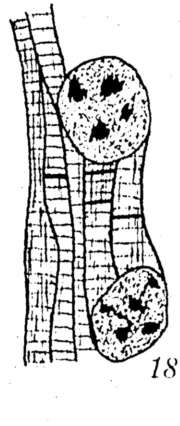

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